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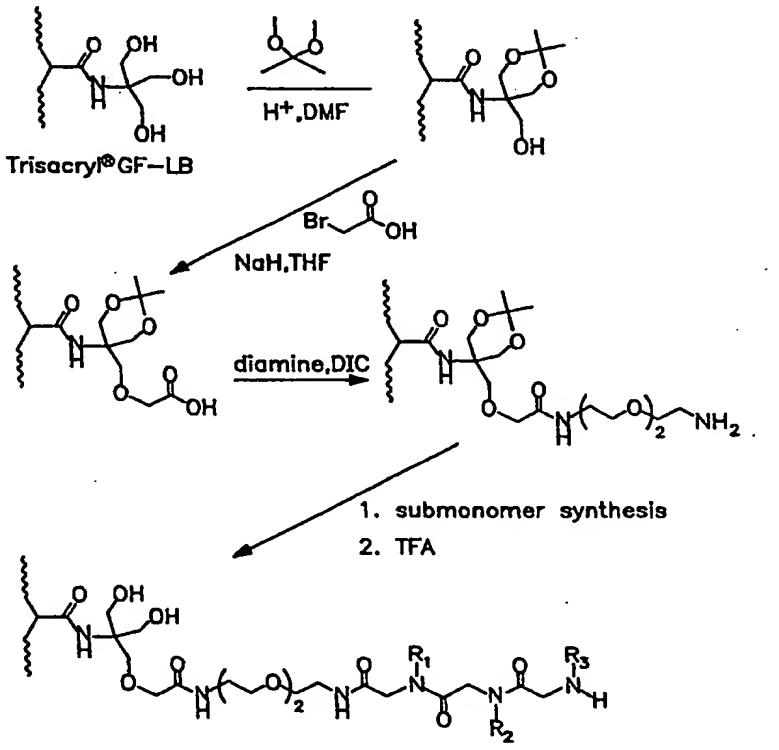
(57) Abstract

Novel functionalized substrates are provided having a surface which is hydrophilic in a first state and hydrophobic in a second state, so that the substrate can be used in either aqueous or organic media. The substrate surface contains a plurality of hydrophilic sites which can be readily protected and deprotected. In use, generally, a fraction of the sites are protected, leaving the remainder available for participating in organic synthetic processes to be conducted using organic reagents and solvents, e.g., solid phase organic synthesis of ligands which may or may not be oligomeric. Following synthesis, the protected hydrophilic sites are deprotected, regenerating the substrate surface in hydrophilic form for use with aqueous reagents, e.g., in screening and/or separation procedures to be conducted in aqueous media.



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SUBSTRATES USEFUL IN BOTH AQUEOUS AND ORGANIC MEDIA, AND ASSOCIATED METHODS OF PREPARATION AND USE

Technical Field

This invention relates generally to substrates which are useful in a variety of chemical processes, including solid phase chemical synthesis and screening and/or separation methods. More particularly, the invention relates to functionalized substrates which can exist in a first, hydrophobic state, and in a second, hydrophilic state, such that the substrate can be used in either aqueous or organic media. The invention also relates to methods of using the substrates in chemical processes in which organic reagents and solvents are used in certain steps, while an aqueous medium is required in others. The invention further relates to methods for preparing these novel dual-use substrates, and to a reaction apparatus for using them.

Background

Solid supports are necessary in many laboratory procedures involved in chemistry and biotechnology. For example, solid supports are necessary in the solid phase synthesis of organic materials, e.g., ligands such as oligopeptides, oligopeptoids, and oligonucleotides. Solid supports are also used in identifying or quantitating an analyte of interest in a sample solution, which may involve, for example, detecting a label present on the support following an analytical procedure such as a hybridization assay or the like. Generally, a first type of solid support is used in processes which must be carried out in an organic medium, i.e., a support which has a hydrophobic surface and is thus compatible with organic reagents. A second type of solid support, having a hydrophilic surface, must be used in processes which are carried out in an aqueous solution. Thus, if a particular laboratory procedure involves one or more steps to be conducted in an organic solvent, and one or more steps to be conducted in an aqueous medium, different supports must be used.

One application wherein substrates are used in a multi-step process involving both organic and aqueous media is wherein the substrate is first used in solid phase organic synthesis, and the support-bound synthesized materials are then used in an aqueous medium, e.g., in aqueous screening or separation procedures. Currently, a first, hydrophobic support is used in the solid phase synthesis step which is conducted using organic reagents and solvents. The support-bound organic materials so prepared are then cleaved from the first support and reattached to a second, hydrophilic support to enable use of the support-bound compounds in an aqueous medium, e.g., in subsequent screening and/or separation processes. This is at present what is done in the preparation and use of combinatorial libraries. That is, in combinatorial processes, a plurality of chemically distinct organic ligands are synthesized on a hydrophobic solid support using organic reagents and an organic medium. The synthesized ligands are cleaved and reattached to a hydrophilic support, and then used in a screening procedure to identify a particular component of interest, a process which is typically carried out in an aqueous medium. As combinatorial processes involve multiple repetition of these steps, the cleavage and reattachment procedures are necessarily repeated as well.

The present invention eliminates the need for two types of supports in such processes, wherein organic reagents and solvents are required for certain steps and an aqueous medium is required for others. That is, the inventors herein have now provided a single solid support which is readily converted from a hydrophilic state to a hydrophobic state and vice versa, and thus enables use of a single substrate in chemical and biotechnological processes wherein certain steps are carried out with organic reagents, in an organic medium, and other steps are conducted in an aqueous medium.

The novel substrates, which eliminate the need for two different supports, one hydrophobic and the other hydrophilic, in processes necessitating both organic and aqueous reagents, find use in a variety of contexts, as will be appreciated by those skilled in the art. However, one important application of the invention, as alluded to above, is in the area of combinatorial chemistry. "Combinatorial chemistry" refers to the recently emerging science involving the rapid, automated synthesis of enormous numbers of compounds of known structures, in order to screen for a particular compound or

compound of interest. The dramatic advances in this area of technology have made so-called "molecular diversity" generation, and the associated screening processes, into a fundamentally important area for use in drug research.

A number of methods for systematizing the synthesis of a multiplicity of compounds to screen for pharmacological or biological activity have, accordingly, been developed. Instead of synthesizing individual compounds and testing them, individually, for biological activity, combinatorial chemistry involves simultaneous preparation of a very large number of chemically distinct compounds in a "library" which may contain one or more specific active compounds. This generally involves assembling every possible combination of a given set of chemical "building blocks," e.g., amino acids, nucleotides, or the like, and documenting the identity and order of the individual monomers used. The many support-bound compounds so prepared are then screened using any one of a number of procedures, as will be discussed briefly below, to identify a particular active compound.

By way of background, Houghten (1985) *Proc. Natl. Acad. Sci. USA* 82:5131-5135), describes the preparation of a combinatorial library of peptides using a modification of the Merrifield method (Merrifield (1963) *J. Am. Chem. Soc.* 85:2149-2154; Tam et al., *The Peptides* (New York: Academic Press, 1975), at pp. 185-249). Oligopeptides are synthesized in individual polyethylene bags each containing C-terminal amino acids bound to a solid support; after each sequential amino acid coupling step, the contents of the bags are mixed. In this manner, a plurality of bags each containing different support-bound oligopeptides can be simultaneously deprotected and coupled to the same protected amino acid. The result of the Houghten method is a plurality of polyethylene bags each containing a different peptide sequence. The peptides in each bag may then be recovered and screened for pharmacological or biological activity.

An alternative combinatorial method has been described by Geysen et al. (1984) *Proc. Natl. Acad. Sci. USA* 81:3998-4002; *see also* International Publications Nos. WO 86/0687 and WO 86/00991. The Geysen method is a modification of the Merrifield solid-phase synthesis wherein the C-terminal amino acid residues are bound to solid

supports in the form of polyethylene pins. The pins are treated individually or collectively to sequentially couple additional amino acids thereto to form a plurality of chemically distinct oligopeptides. Without removing the oligomers from the supports, the synthesized oligopeptides can be individually assayed for desired pharmacological activity.

An alternative to isolating and then identifying the active components is to make "overlapping" mixtures in which structurally identical oligomers are present in each of two or more libraries. Here, two or more libraries are made from the same overall set of compounds, but different individual compounds are pooled together in the mixtures. Cross-referencing the active pools between the libraries yields a limited number of candidates for the active components. The strategy of preparing overlapping mixtures was used in Pirrung et al. (1995), *J. Am. Chem. Soc.* 117:1240-1245, for preparing an "indexed" combinatorial library for the deconvolution of 54 carbamates synthesized in solution phase. Smith et al. (1994) *BioMed Chem. Lett.* 4:2821-2824, describes the use of a similar method for screening 1600 esters or amides.

These and other combinatorial methods can be extended to the synthesis of polynucleotide libraries using phosphoramidite or phosphotriester chemistry. See Beaucage et al. (1981) *Tetrahedron Lett.* 22:1859-62, and Itakura et al. (1975) *J. Biol. Chem.* 250:4592 (1975), which describe these now conventional techniques. Libraries of polysaccharides and peptoid polymers may be prepared as well.

Because of the tremendous opportunity for generating and identifying and/or separating potentially important biomolecules using diverse libraries, there is a need to develop better and more versatile materials as well as simpler methods to exploit the potential of this field of research. The present invention makes an important contribution to the art by providing supports which simplify synthesis and separation processes, particularly synthesis and use of combinatorial libraries. The invention eliminates the need for repeated cleavage and reattachment steps to accommodate the use of two types of supports, and thus substantially reduces the time and complexity involved in the synthesis and use of combinatorial libraries.

While the invention is thus extremely useful in the area of combinatorial chemistry, it is also, as explained above, useful in a number of contexts wherein it is desired to use a single solid support in both organic solvents and aqueous solvents. It should also be emphasized that in its hydrophilic state, the novel substrate is useful as a chromatographic support and can be used in the screening and separation, in an aqueous medium, of a variety of aqueous-soluble biomolecules. An additional use of the novel substrate is in fractionating proteins, wherein a mixture of proteins is synthesized on the substrate surface (in organic media), a retained protein of interest is identified (using a screening procedure requiring an aqueous medium), and the protein of interest is resynthesized on the substrate.

Summary of the Invention

Accordingly, it is an object of the present invention to address the above-mentioned need in the art by providing a novel substrate alternatively useful in processes involving organic reagents and solvents and in procedures which are carried out in an aqueous medium.

It is another object of the invention to provide such a substrate which is useful in solid-phase organic synthesis and, subsequently, in screening and/or separation processes which are conducted in an aqueous medium.

Another object of the invention is to provide a single substrate on which a library of chemically distinct ligands may be synthesized and then used in combinatorial processes.

Still another object of the invention is to provide a method for conducting a first chemical procedure involving organic reagents and a second chemical procedure involving an aqueous medium, using a substrate as provided herein.

Further objects of the invention derive from additional uses of the novel substrates, e.g., as chromatographic supports, as substrates useful in protein fractionation processes, and the like.

Yet another object of the invention is to provide a method for making the novel substrates.

Yet a further object of the invention is to provide a reaction apparatus for using the novel substrates, and (i) a method for screening a library of compounds, and (ii) a chromatographic separation method involving the novel apparatus.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

In one embodiment of the invention, then, a substrate is provided having a surface that is hydrophilic in a first state and hydrophobic in a second state. The substrate surface at the outset has a plurality of hydrophilic sites, e.g., hydroxyl groups, carboxyl groups, thiol groups, or the like. A subset of these hydrophilic sites is protected using conventional protecting groups and techniques, and the remaining, unprotected hydrophilic sites are then used for reaction. That is, the unprotected sites are caused to react with either (1) a preformed ligand, (2) an initial species which is chemically modified on the support, or (3) a monomeric unit which serves to bind additional monomers, in succession, in the synthesis of support-bound oligomeric ligands. At this point, then, the substrate surface is hydrophobic. When it is desired to return the substrate to a hydrophilic state, the protecting groups are removed.

In a preferred embodiment, an initial substrate-bound monomer serves as the first of a number of monomers in the synthesis of an oligomer, e.g., an oligonucleotide, an oligopeptide, an oligopeptoid, or the like. As above, after oligomer synthesis is complete, the protecting groups are removed to regenerate the substrate surface in hydrophilic form, so that the support can then be used procedures requiring an aqueous medium. Such procedures include: screening processes to identify a particular support-bound ligand of interest (e.g., a pharmaceutically active compound), particularly, although not necessarily, using combinatorial techniques; separation and removal of a component of interest in a sample, i.e., by binding that component to the support-bound ligands; electrophoretic processes, including capillary electrophoresis; spectroscopic detection strategies, including mass spectrometric analysis; and a variety of other analytical methods, as will be appreciated by those skilled in the art.

In combinatorial processes, each the support-bound ligands are each chemically distinct, so as to serve as a combinatorial library. The library, which is an essence of a plurality of support-bound oligomeric ligands, can be used immediately following synthesis, in screening and separation processes. That is, the combinatorial library as synthesized on the novel substrates can be used without need for a second support or intermediate cleavage and reattachment steps, as required in conventional methods, i.e., wherein ligands are cleaved from the first, hydrophobic substrate on which they are synthesized, and reattached to a second, hydrophilic substrate, for use in subsequent procedures to be carried out in aqueous media.

Thus, the invention avoids the inconvenience, expense, and additional time involved in procedures previously requiring two different solid supports, one hydrophobic and thus compatible with organic reagents and solvents, and the other hydrophilic and thus compatible with aqueous media. Applicants have now provided a multi-functional substrate which eliminates the need for separate hydrophobic and hydrophilic supports in processes involving both organic and aqueous media, e.g., combinatorial synthesis and screening procedures, protein fractionation processes, and the like. The substrates are also useful in certain processes wherein only a hydrophilic surface is desired, e.g., as chromatographic supports, so that the surface hydrophilic groups will be in deprotected form. Similarly, the substrates may be used in processes wherein only a hydrophobic surface is necessary, e.g., solid phase organic synthesis, and wherein there is no need to render the surface compatible with aqueous media thereafter.

Detailed Description of the Figures

Figure 1 is a generalized schematic illustration of the dual use of the substrate of the invention for use in a two-step process requiring organic reagents and solvents, e.g., organic synthesis on the substrate surface, and subsequent use of the support-bound organic materials in an aqueous solution, e.g., in screening a crude mixture of proteins in water.

Figure 2 schematically illustrates a specific embodiment of the invention wherein Trisacryl® resin provides the hydrophilic groups to be protected and deprotected on the substrate surface.

Figure 3 schematically illustrates use of the novel substrates as chromatography supports, wherein solid phase synthesis on an agarose support is followed by affinity chromatography and electrophoretic screening.

Detailed Description of the Invention

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular substrate materials, synthesis and protection techniques, screening and separation processes, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

It must be noted that, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include the plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a ligand" includes mixtures of two or more ligands, reference to "an oligomer" includes mixtures of two or more oligomers, reference to "a protecting group" may include mixtures of protecting groups, and the like.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

The term "functionalized substrate" as used herein relates to a solid support having a plurality of functional groups on the support surface. In the present case, the substrate in its unmodified, unprotected state is "functionalized" by virtue of having a plurality of hydrophilic groups on the substrate surface.

The term "dual use" as used herein refers to utility in two distinct types of chemical processes involving different reagents, techniques and products, i.e., (1) procedures carried out using organic reagents and solvents, such as solid-phase organic synthesis, and (2) procedures carried out in an aqueous medium, such as screening and separation processes using support-bound organic ligands.

The term "monomer" as used herein refers to a chemical entity that can be covalently linked to one or more other such entities to form an oligomer. Examples of "monomers" include amino acids, nucleotides, saccharides, peptoids, and the like. In general, the monomers used in conjunction with the present invention have first and second sites (e.g., C-termini and N-termini, or 5' and 3' sites) suitable for binding to other like monomers by means of standard chemical reactions (e.g., condensation, nucleophilic displacement of a leaving group, or the like), and a diverse element which distinguishes a particular monomer from a different monomer of the same type (e.g., an amino acid side chain, a nucleotide base, etc.). The initial support-bound "monomer" is generally used as a building-block in a multi-step synthesis procedure, conducted in a nonaqueous medium, to form a complete ligand, such as in the synthesis of oligopeptides, oligopeptoids, oligonucleotides, and the like.

As used herein, the term "amino acid" is intended to include not only the L-, D- and nonchiral forms of naturally occurring amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine), but also modified amino acids, amino acid analogs, and other chemical compounds which can be incorporated in conventional oligopeptide synthesis, e.g., 4-nitrophenylalanine, isoglutamic acid, isoglutamine, -nicotinoyl-lysine, isonipecotic acid, tetrahydroisoquinoleic acid, -aminoisobutyric acid, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, -alanine, 4-aminobutyric acid, and the like.

"Molecular mimetics" include, but are not limited to, small organic compounds; nucleic acids and nucleic acid derivatives; saccharides or oligosaccharides; peptide mimetics including peptides, proteins, and derivatives thereof, such as peptides containing non-peptide organic moieties, synthetic peptides which may or may not contain amino acids and/or peptide bonds, but retain the structural and functional features of a peptide ligand, and peptoids and oligopeptoids which are molecules comprising N-substituted glycine, such as those described by Simon et al., *Proc. Natl. Acad. Sci. USA*

89:9367 (1992); and antibodies, including anti-idiotype antibodies. Methods for the identification and production of molecular mimetics are described more fully below.

A "peptoid" is a polymer made up, at least in part, of monomer units of "amino acid substitutes" which substitutes are any molecule other than an amino acid, but which serves in the peptoid polymer to mimic an amino acid. Particularly preferred monomer units are N-alkylated derivatives of glycine. Peptoids are produced by linking the "amino acid substitutes" into a linear chain or cyclic structure with amino acids and/or other amino acid substitutes. The links may include, without limitation, peptide bonds, esters, ethers, amines, phosphates, sulfates, sulfites, thioethers, thioesters, aliphatic bonds, and carbamates. Examples of amino acid substitutes include, without limitation, N-substituted glycine, N-alkylated glycines, N-substituted alanine, N-substituted D-alanine, urethanes, substituted hydroxy acids, such as hydroxyacetic acid, 2-hydroxypropanoic acid, 3-hydroxypropanoic acid, 3-phenyl-2-hydroxypropanoic acid, and the like. A peptoid may comprise amino acid substitutes using more than one type of link provided the chemistry for the reaction schemes are compatible and encompassed generally by the reactions described herein. Other examples of amino acid substitutes and peptoids are described in Bartlett et al., PCT WO91/19735 and Zuckermann et al., PCT WO94/06451.

The terms "conventional" and "naturally occurring" as applied to peptides herein refer to polypeptides, also referred to as proteins, constructed only from the naturally occurring amino acids: Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp and Tyr.

The terms "nucleoside" and "nucleotide" are intended to include those moieties which contain not only the known purine and pyrimidine bases, but also other heterocyclic bases which have been modified. Such modifications include methylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles. In addition, the terms "nucleoside" and "nucleotide" include those moieties which contain not only conventional ribose and deoxyribose sugars, but also other sugars as well. Modified nucleosides or nucleotides will also include modifications on the sugar moiety, e.g., wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or are functionalized as ethers, amines, or the like.

The term "saccharide" is intended to include not only naturally occurring mono- and di-saccharides, but also modified saccharides. Examples of monosaccharides include trioses, such as glyceraldehyde and dihydroxyacetone, tetroses, such as erythrose, erythrulose and threose, pentoses, such as ribose, ribulosem arabinose, xylose, xylulose and lyxose, hexoses, such as allose, altrose, glucose, mannose, gulose, idose, galactose, talose, psicose, fructose, sorbose, and tagatose, heptoses, such as sedoheptulose, and the like. Disaccharides include dimers of the any of the above monosaccharides attached by way of α-1,2, α-1,3, α-1,4, α-1,6, β-1,2, β-1,3, β-1,4, β-1,6 linkages, or the like. Examples of such disaccharides include maltose, lactose, sucrose, and the like. Modified saccharides include those wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or are functionalized as ethers, amines, phosphates, or the like.

The term "oligomer" is used herein to indicate a chemical entity that contains a plurality of monomers. As used herein, the terms "oligomer" and "polymer" are used interchangeably, as it is generally, although not necessarily, smaller "polymers" that are prepared using the method of the invention, particularly in conjunction with combinatorial chemistry techniques. Examples of oligomers and polymers include polydeoxyribonucleotides, polyribonucleotides, other polynucleotides which are N- or C-glycosides of a purine or pyrimidine base, polypeptides, polysaccharides, and other chemical entities that contain repeating units of like chemical structure. In the practice of the instant invention, oligomers will generally comprise about 2-50 monomers, preferably about 2-20, more preferably about 3-10 monomers.

The term "ligand" as used herein refers to moieties which are capable of covalently or otherwise chemically binding a compound of interest. Typically, when the present substrates are used in solid phase synthesis, they are used so that "ligands" are synthesized thereon. These solid-supported ligands can then be used in screening or separation processes, or the like, to bind a component of interest in a sample. The term "ligand" in the context of the invention may or may not be an "oligomer" as defined above. However, the term "ligand" as used herein may also refer to a compound that is not synthesized on the novel substrate, but that is "pre-synthesized" or obtained commercially, and then attached to the substrate.

The term "sample" as used herein relates to a material or mixture of materials, typically, although not necessarily, in fluid form, containing one or more components of interest.

The term "analyte" as used herein refer to a component of interest in a sample, to be isolated and identified, and may include biomolecules or non-biomolecules capable of binding to a ligand. Analytes can include, for example, oligonucleotides, receptors, pathogens, enzymes and the like. In the context of the present invention, analytes are essentially "ligates" which bind to the support-bound ligands.

The terms "protection" and "deprotection" as used herein relate, respectively, to the addition and removal of chemical protecting groups using conventional materials and techniques within the skill of the art and/or described in the pertinent literature; for example, reference may be had to Greene et al., Protective Groups in Organic Synthesis, 2nd Ed., New York: John Wiley & Sons, 1991. Protecting groups prevent the site to which they are attached from participating in the chemical reaction to be carried out. In the context of the invention, a subset of hydrophilic sites initially present on the solid support are protected to prevent reaction of those sites and temporarily render the support hydrophobic. Methods and conditions for the removal of protecting groups are well known in the art and described, for example, in Greene et al., cited above. Suitable methods for removing hydroxyl protecting groups, in particular, include, but are not limited to, treatment with an acid of sufficient strength to remove the protecting group but which will not otherwise alter the properties of the solid support or any components

bound thereto. After deprotection, the substrate is again hydrophilic and compatible with aqueous solvents.

The terms "reactive hydrophilic site" or "reactive hydrophilic group" refer to hydrophilic moieties which can be protected with standard organic protecting groups and used as the starting point in a synthetic organic process. This is contrast to "inert" hydrophilic groups which could also be present on a support surface, e.g, hydrophilic sites associated with polyethylene glycol, a polyamide or the like.

The term "compatible" as in a support surface which is "compatible" with aqueous media or "compatible" with organic media, means the particular surface will facilitate and not hinder a desired reaction, nor will the surface interact with reaction components, reagents, solvents, or the like, in a deleterious manner. Generally, this means that a surface is "compatible" with a medium if reagents or compounds in the medium are capable of interacting and/or reacting with the surface, i.e., chemical reactions can take place on the surface in the compatible medium. For example, a hydrophilic surface is "compatible" with an aqueous medium because there will be hydrogen-bonding interaction facilitating reaction at the surface in an aqueous medium. "Compatibility" of a hydrophilic surface also implies that the surface is "wettable" with aqueous reagents, and that any hydrophilic interior, as with porous substrates will be wettable and compatible with an aqueous medium as well. In general, the term "hydrophilic surface" is used to include the hydrophilic interiors of porous substrates where reactions might be taking place as well. This is particularly true with porous supports that may have swelled in water. When a hydrophobic surface is placed in water, by contrast, there might be shrinkage and a consequent loss of surface area. A hydrophobic surface is in the same sense "compatible" with organic reagents and solvents, in that reaction at the surface is supported by virtue of the "compatibility," but the reaction is of a different type, generally involving nonpolar interaction, as in solid phase organic synthesis. One final point to be made with respect to hydrophilic surfaces, hydrophobic surfaces, and compatibility, is that hydrophilic surfaces normally bear surface polar groups which are capable of forming hydrogen bonds, and thus display greater binding with respect to aqueous or other hydrophilic materials (such that there is

"compatibility" between the surface and such materials) but will generally display low binding to hydrophobic moieties (and are thus "incompatible" therewith), and, analogously, hydrophobic surfaces generally display greater binding to hydrophobic moieties and low binding to hydrophilic moieties.

The term "aqueous medium" as used herein is used to refer to a liquid medium containing less than about 50 vol.% organic solvents, preferably less than 25 vol.% organic solvents, more preferably less than about 20 vol.% organic solvents, still more preferably less than about 15 vol.% organic solvents, and most preferably less than about 10 vol.% organic solvents. Correspondingly, the term "organic medium" as used herein is used to refer to a liquid medium containing less than about 50 vol.% aqueous liquids, preferably less than about 25 vol.% aqueous liquids, more preferably less than about 20 vol.% aqueous liquids, still more preferably less than about 15 vol.% aqueous liquids, and most preferably less than about 10 vol.% aqueous liquids. Organic solvents include, for example, acetonitrile, dimethylformamide, methylene chloride, tetrahydrofuran, dimethylsulfoxide, and the like.

Accordingly, the invention in a first embodiment is directed to a dual use substrate, i.e., a substrate which is compatible with organic reagents and solvents in a first state and aqueous solvents in a second state. The substrate comprises a solid support having a surface bearing a plurality of hydrophilic sites, wherein: (i) protection of a subset of the hydrophilic sites renders the support useful for covalent attachment of a ligand or solid-phase organic synthesis of a ligand, in an organic solvent; and (ii) deprotection of the subset regenerates the subset in hydrophilic form, such that the support having ligand bound thereto then exists in a hydrophilic state. When the functionalized substrate is in the "first state," then, the subset of hydrophilic sites is protected and the remaining sites are unprotected: the substrate is then hydrophobic and may be used in processes requiring organic reagents and solvents, such as synthesizing organic ligands on the substrate at the unprotected sites. When the functionalized substrate is in the "second state," the formerly protected subset of hydrophilic sites is no longer protected, and the substrate, generally having organic materials such as oligomeric ligands bound thereto, is again hydrophilic. The organic materials bound to the support

may be complete ligands that were covalently attached, or, oligomeric or other types of ligands that were synthesized on the support using solid-phase organic synthetic techniques. The "dual use" concept of the invention is illustrated schematically in Figure 1.

The substrate may comprise any material which has a plurality of hydrophilic sites or which can be treated or coated so as to provide a plurality of hydrophilic sites on the substrate surface. Suitable materials include, but are not limited to, supports which are typically used for solid-phase chemical synthesis, e.g., cross-linked polymeric materials. These include cross-linked divinylbenzene styrene-based polymers, including divinylbenzenehydroxymethylstyrene copolymers, divinylbenzene-chloromethylstyrene copolymers, and divinylbenzene-benzhydrylaminopolystyrene copolymers. Alternative substrates include agarose supports (e.g., Sepharose®) and dextran supports (e.g., Sephadex®), as well as supports made of cellulosic polymers, polysaccharides, polyacrylamides, silica, glass, and the like. The supports may be obtained commercially and used as is, or they may be treated or coated for use in conjunction with the invention (i.e., to include a plurality of hydrophilic groups thereon).

The hydrophilic groups are generally neutral under experimental conditions. Preferably, experimental conditions involve neutral pH, but may include acidic or basic conditions as well. The hydrophilic groups may be hydroxyl groups, carboxyl groups, thiol groups, substituted or unsubstituted amines, or the like, but are preferably hydroxyl groups. These may be inherently present in the material used for the solid support, or they may be provided by treating or coating the support with a suitable material. For example, the surface may be treated with polyethylene glycol or with Trisacryl® resin, such that a plurality of hydroxyl groups is then provided on the surface. The hydrophilic sites may also be provided by treating the surface so that hydrophilic groups such as hydroxyl groups or the like are bound to the support surface through a "linker," i.e., a chemical tethering moiety. It is preferred that the hydrophilic moieties be physically grouped so that a simple chemical reaction with a single protecting group will render the substrate surface hydrophobic. For example, as may be seen in Figure 2, Trisacryl® resin provides individual groups of three hydroxyl moieties bound through an amide

linkage to the substrate surface. Using a protecting group which binds to two out of the three hydroxyl moieties (such as 2,2-dimethoxypropane, as shown) thus "ties up" two out of the three hydroxyl moieties, while leaving the third hydroxyl group unprotected and thus capable of use in chemical reactions as desired, e.g., to synthesize oligomeric compounds thereon, to bind complete ligands, or the like. Alternative, but substantially equivalent, materials can be used as well, wherein individual groups of hydroxyl moieties (or other hydrophilic moieties) are provided and somewhat isolated from each other, at least to the extent that a single protecting reagent can bind the majority but not all of the unprotected hydroxyl groups. For example, the Trisacryl® surface shown in Figure 2 can be replaced with a support-bound monosaccharide moiety similarly bound to the substrate through a linker, such as an amide.

Finally, it is also possible to work with a substrate comprised of a material which on its surface has a plurality of substantially evenly distributed hydrophilic groups, or with a substrate which has been treated or coated so as to have a plurality of substantially evenly distributed surface hydrophilic groups. The amount of protecting reagent used is selected so that, relative to the surface hydrophilic groups, approximately 50% to 95% of the hydrophilic groups are then protected, preferably approximately 60% to 80%, and most preferably approximately 70% to 80%.

To provide the substrate in its hydrophobic state, then, so as to be compatible with organic solvents, a subset of the hydrophilic sites is protected as just described, enabling reaction of the unprotected sites, typically with organic, hydrophobic moieties. The size of the subset, i.e., the fraction of hydrophilic sites which is protected, is selected so as to ensure that after reaction of the unprotected sites and deprotection of the protected sites, a sufficient number of unprotected hydrophilic sites will be regenerated so that the support then exists in a hydrophilic state. As noted above, this means that the protected subset will represent approximately 50% to 90%, and preferably on the order of 60% to 90%, of the initially present hydrophilic groups. As explained above, protection and deprotection are carried out using conventional reagents and means (see Greene et al., *supra*). The fraction of hydrophilic sites which are to be protected can readily be

controlled by adjusting the quantity of protecting reagent used to correspond to the fraction of hydrophilic sites to be protected.

Any number of protecting groups can be used, as will be appreciated by those skilled in the art. Again, reference may be had to Greene et al., although suitable protecting groups will be known to or easily deduced by those working in the field of synthetic organic or bio-organic chemistry. The only requirements for the protecting groups used herein are that: (1) they be "orthogonal" so as to remain in place during other chemical syntheses or procedures which are carried out on the unprotected sites, e.g., coupling of amino acids, peptide mimetics, nucleotides, and the like; and (2) they are compatible with whatever temperatures, reaction conditions and reagents are employed while they are in place, i.e., are not degraded, chemically altered, or removed from the protected site. Generally, although not necessarily, the protecting groups are acid-cleavable. Examples of suitable protecting groups include, but are not limited to: (a), for diol protection, 2,2-dimethoxypropane, acetals such as benzylidene acetal and *p*-methoxybenzylidene acetal, bifunctional silyl ethers such as di-*t*-butylsilylene, and compounds which upon reaction with a 1,2-diol will form acetonides, cyclic carbonates or cyclic boronates; and (b) for protection of a single hydroxyl site, (i) protecting groups which will give rise to ethers, e.g., tetrahydropyranyl, dihydropyranyl, trimethylsilyl, substituted or unsubstituted benzyl (if substituted, typically with electron withdrawing groups such as NO_2), and triphenylmethyl, and (ii) protecting groups which will give rise to esters, such as acetyl, trifluoroacetyl, and trichloroacetyl.

After the desired fraction of hydrophilic sites has been protected, the remainder of the sites, i.e., the unprotected sites, are used to covalently attach a ligand, or to provide the starting point for the synthesis of a ligand which may or may not be oligomeric. Preferred ligands include oligopeptides, oligonucleotides, oligosaccharides, oligomers of peptide mimetics such as oligopeptoids, and the like. Oligopeptides are particularly useful ligands herein as they provide for a direct approach to affinity chromatography, and single amino acids can be used as well. A further advantage to immobilized peptide ligands is that they are relatively stable and can be reused many times. They are versatile because either the amino or the carboxyl terminus of the peptide can be blocked in order

to lessen the possibility of proteolysis by exopeptidases during affinity chromatography. Methods and devices for the manufacture of peptides or families thereof which have a specific target-binding property are described in, for example, U.S. Patent No. 5,010,175 to Rutter et al. and U.S. Patent No. 5,240,680 to Zuckermann et al. Oligomers of peptide mimetics, and particularly oligopeptoids, have additional advantages, as oligopeptoids can be synthesized so that they are highly stable and not susceptible to enzymatic degradation.

In using the unprotected hydrophilic sites of the partially protected substrate in solid phase chemical synthesis, conventional reagents and methods for making oligopeptides, oligopeptoids, oligonucleotides, and the like, can be used. In combinatorial processes, it is the materials and techniques now used in combinatorial chemical techniques which are employed, which are known in the art and discussed, for example, in the Houghten, Geysen et al. (1984), Geysen et al. (1986), Tjoeng et al., Beaucage et al., and Itakura et al. references cited earlier herein.

After covalent attachment or solid phase synthesis of the ligand using the unprotected hydrophilic sites on the substrate surface, the protected hydrophilic groups are then deprotected using cleavage reagents appropriate to the selected protecting groups. After deprotection, the substrate surface is once again rendered hydrophilic, but now has the aforementioned ligands bound thereto. In this state, the substrate may be used in any process which involves the use of support-bound ligands in an aqueous medium, e.g., screening and separation processes, wherein a specific support-bound ligand of interest is isolated and identified, or wherein the support-bound ligands are used to bind a component of interest in a sample, which may then be isolated and identified.

Referring again to Figure 2 for purposes of exemplification, the functionalized substrate shown is Trisacryl®, which, as alluded to above, provides groups of three hydroxyl moieties linked to the support surface through a single amide bond. At the outset, the substrate surface is completely hydrophilic as all of the hydroxyl groups are unprotected. Two of every three hydroxyl groups are then protected using 2,2-dioxypropane in the presence of acid and an organic solvent such as dimethylformamide

(DMF). The third hydroxyl in each group is then used as a site for solid phase organic synthesis. In the example illustrated in Figure 2, the unprotected hydroxyl groups are treated with bromoacetic acid in the presence of sodium hydride to convert the free hydroxyl group to a carboxyl group, which is then further derivatized with a diamine linker in the presence of N,N'-diisopropylcarbodiimide (DIC), followed by oligopeptide synthesis (termed "submonomer synthesis" in the figure) on the free amine. Although not explicitly shown in Figure 2, oligopeptide synthesis, as will be appreciated by those skilled in the art, involves sequential addition of carboxyl-protected amino acids to a growing peptide chain, with each additional amino acid in the sequence similarly protected and coupled to the terminal amino acid of the oligopeptide under conditions suitable for forming an amide linkage.

After oligopeptide synthesis is complete, acid (e.g., trifluoroacetic acid, or "TFA," as shown in Figure 2) is used to remove the hydroxyl protecting groups along with the protecting groups used on the individually added amino acids, e.g., *t*-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), benzyloxycarbonyl (Cbz), *p*-toluenesulfonyl (Tos), 2,4-dinitrophenyl, benzyl, biphenylisopropyloxycarboxycarbonyl, cyclohexyl, isopropyl, acetyl, *o*-nitrophenylsulfonyl, and the like. The various R substituents in the product shown in Figure 2 (i.e., R^1, R^2 and R^3) represent amino acid side chains. Removal of protecting groups regenerates a hydrophilic surface. The support-bound oligopeptides provided can then be used in aqueous media, e.g., in screening procedures involved in combinatorial processes, in chromatographic methods, in protein fractionation, and the like. In fractionating proteins using the techniques and substrate of the invention, a mixture of support-bound oligopeptides is synthesized using a solid-phase synthesis technique such as described above, the support-bound oligopeptides are then screened, in an aqueous medium, to identify a particular oligopeptide of interest, and that oligopeptide is then resynthesized on the support, using organic reagents and solvents as explained above.

Similar procedures are followed in synthesizing support-bound ligands of other types. That is, a subset of hydrophilic groups is protected and the remaining hydrophilic groups are used for attachment or synthesis of a ligand, a process which is carried out

using organic reagents and solvents. At this point, the substrate surface is hydrophobic, and the support-bound ligands can be used in any of a variety of processes requiring organic reagents and/or solvents. The protecting groups on the surface hydrophilic moieties may at any time be removed, along with protecting groups present on the ligands (if any), and the regenerated hydrophilic surface can then be used in any process necessitating aqueous reagents and an aqueous medium.

The method of using the novel substrates to prepare and use a combinatorial library involves the same general procedures outlined above with respect to partial protection of the hydrophilic surface to render the substrate hydrophobic, synthesis of support-bound ligands using organic reagents and solvents, followed by deprotection and use of the support-bound ligands in processes requiring an aqueous medium. For purposes of completeness, however, the following is included as a description of the steps which would be used to carry out synthesis and use of a combinatorial library using the substrates and general methods of the invention. First, a plurality of reaction vessels is provided each containing a substrate as described herein. A predetermined subset of the hydrophilic sites present on the substrate surface is then protected so as to negate the hydrophilicity of those sites. A different monomeric entity, each capable of binding to the unprotected hydrophilic sites, is distributed into each of the reaction vessels, such that an initial support-bound monomer is provided in each vessel. Additional monomers are coupled to the growing oligomer chain, with the identity and order of monomers documented to enable synthesis of a plurality of support-bound, chemically distinct oligomers. This last step may involve a "split/mix" approach, wherein after every monomer addition, the contents of the reaction vessels are alternatively divided and mixed in a way that provides for a completely diverse set of ligands (*see, e.g.*, Pirrung et al.). The distinct oligomers in the combinatorial library so provided are then screened for activity, generally by screening individual sublibraries containing mixtures of distinct oligomers, identifying active sublibraries, and then determining the oligomeric compounds of interest by generating different sublibraries and cross-correlating the results obtained.

References describing construction of small organic molecule libraries include: Thompson et al., *Chem. Rev.* 96:555-600 (1996); Gallop et al., *J. Med. Chem.* 37:1233-1251 (1994); and Gordon et al., *J. Med. Chem.* 37:1385-1401 (1994). A reference related to mimotopes and describing the construction of peptides on solid supports is U.S. Patent No. 4,708,871 to Geysen et al., while other references generally describing construction of peptoid libraries include Bartlett et al., PCT Publication No. WO91/19735, and Zuckermann et al., PCT Publication No. WO94/06451. References describing screening of compounds and determination of sequences include U.S. Patent Nos. 4,833,092 to Geysen et al., 5,194,392 to Geysen et al., 5,573,905 to Lerner et al., and 5,585,277 to Bowie et al.

As alluded to earlier, the novel substrates are not limited to use in combinatorial chemistry, but find use in a wide variety of contexts, including separation and removal of a component of interest in a sample, i.e., by binding that component to the support-bound ligands (e.g., with respect to oligonucleotides, this step could involve hybridization and the use of labeled oligomers), electrophoresis, and spectroscopic detection. Along these lines, Figure 3 illustrates use of the present substrate in a different context. As may be seen in the Figure, the same agarose substrate, present in all of the reaction vessels, is used for equimolar mixture synthesis, and can then be used, directly, in affinity chromatographic and electrophoretic screening. Other screening methods can also be used. e.g., in ELISA procedures, in Lowry assays for determining protein concentration in a sample, and the like. The solid phase synthesis is carried out in an organic medium, after partial protection of surface hydrophilic groups to render the substrate hydrophobic, with the latter steps, chromatography and electrophoresis, carried out in an aqueous medium after removal of the protecting groups, such that the surface is in regenerated hydrophilic form.

Thus, combinatorial processes and chromatographic applications represent important fields of use for the present invention. In chromatographic applications, the invention is useful in at least the following contexts: (i) in affinity chromatography with the generation of receptor/molecule specific non-biological ligands on the substrate using solid-phase organic chemistry; and (ii) in the generation of a family of non-biological

ligands on a support to create more general purpose derivatized supports for chromatographic separation of biological and non-biological molecules. With respect to combinatorial processes, coupling combinatorial synthesis and combinatorial library deconvolution strategies, using a single solid support, is also an important application of the invention In other processes as well, the invention simplifies synthesis and subsequent use of support-bound materials. Although combinatorial chemistry and chromatographic applications has been emphasized herein, it will be appreciated by those skilled in the art that the novel substrates will find utility in a host of applications.

It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, that the foregoing description as well as the examples which follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

All patents and publications mentioned herein, both *supra* and *infra*, are hereby incorporated by reference.

Example 1

Preparation of amino-derivatized protected solid support:

Trisacryl GF-LB (100 mL settled volume) was washed with water (2 x 300 mL), ethanol (2 x 100 ml) and ether (3 x 300 mL). The resin was air-dried for 30 min. on a vacuum manifold, and dried overnight in a drying pistol over phosphorus pentoxide at 50 C. The resin was then suspended in a solution of dry tetrahydrofuran (THF, 250 mL) containing 2,2-dimethoxypropane (30 mL, 250 mmol) and p-toluenesulfonic acid (3 g). The suspension was agitated at room temperature overnight under nitrogen. The resin was then drained and washed with THF (3 x 300 mL) and dried *in vacuo* to afford acetonide-protected resin.

The acetonide protected resin was suspended in dry THF (250 mL) to which sodium hydride was added (200 mmol, 4.8g). The suspension was mixed for 10 min. which was followed by the addition of chloroacetic acid. The mixture was heated to reflux for 6 hr., after which the resin was washed with THF (3 x 300 mL), neutralized

with 0.1 N acetic acid in methanol, followed by washing with dichloromethane (2 x 300 mL).

The carboxymethylated resin was then coupled to a diamine linker by suspending the resin in dichloromethane (250 mL) containing 2,2'-(ethylene-dioxyl)bis(ethylamine) (19 g, 125 mmol) and diisopropyl-carbodiimide (23 ml, 150 mmol). The reaction mixture was mixed at room temperature for 6 hr., followed by washing with dichloromethane (3 x 300 mL), and drying *in vacuo* to yield the amino-derivatized protected resin.

Example 2

Preparation of support-bound ligand:

The amino-derivatized protected resin prepared in Example 1 (200 mg) was suspended in dimethylformamide (3 mL), and drained. A peptoid ligand was synthesized. The resin was acylated by adding to the resin 830 µL of 0.6 M bromoacetic acid in DMF, followed by 200 µL of 3.2 M DIC in DMF. This solution was agitated for 30 minutes at room temperature and then drained. This step was then repeated a second time. The resin was then washed with DMF (2 x 2 mL) and DMSO (1 x 2 mL). To the washed resin was added 1.0 mL of a 1 M solution of benzylamine in DMSO. This solution was agitated for two hours at room temperature and then drained. The resin was then washed with DMSO (2 x 2 mL) and DMF (1 x 2 mL). Two additional cycles of acylation and displacement with benzylamine were performed to synthesize a tri-(N-benzyl)glycine on the protected resin. The resin was washed with dichloromethane (3 x 2 mL).

The amino-derivatized resin can also be used for the synthesis of any organic compounds requiring an amine handle. The resin can also be used for the synthesis of combinatorial libraries by using the resin-splitting method.

Example 3

Deprotection of ligand-support:

The peptoid-trimer support was deprotected by the addition of 2 mL of 95% trifluoroacetic acid/water with mixing at room temperature for 20 minutes. The resin was then washed with methanol (2 x 2 mL) followed by water (3 x 2 mL). The hydrophilic resin so prepared can be used in aqueous separations in a similar fashion to crosslinked agarose supports and the like.

CLAIMS

1. A method for conducting chemical processes involving a first procedure which is conducted in an organic medium and a second procedure which is conducted in an aqueous medium, comprising:

(a) providing a solid support having a surface bearing a plurality of reactive hydrophilic moieties, such that the support surface is itself hydrophilic and thus compatible with aqueous media;

(b) protecting a fraction of the reactive hydrophilic moieties while leaving the remaining hydrophilic moieties unprotected, to provide the surface in a hydrophobic form so that it is compatible with organic media;

(c) conducting said first procedure in an organic medium using the hydrophobic surface provided in step (b);

(d) deprotecting the protected fraction of reactive hydrophilic moieties to regenerate the surface in a hydrophilic form; and

(e) conducting said second procedure in an aqueous medium using the hydrophilic surface provided in step (d).

2. The method of claim 1, wherein, in step (c), the first procedure is conducted using the unprotected hydrophilic moieties on the support surface.

3. The method of claim 1, wherein the reactive hydrophilic moieties are selected so that they are uncharged at neutral pH.

4. The method of claim 3, wherein the hydrophilic groups are selected from the group consisting of hydroxyl, carboxyl, thiol, amines, and mixtures thereof.

5. The method of claim 4, wherein the hydrophilic groups are hydroxyl groups.

6. The method of claim 1, wherein the solid support is comprised of a crosslinked polymeric material functionalized to provide the reactive hydrophilic moieties.

7. The method of claim 1, wherein the first procedure comprises covalently binding an organic compound to the unprotected hydrophilic moieties.

8. The method of claim 7, wherein the organic compound is a ligand.

9. The method of claim 7, wherein the organic compound is a monomer capable of further reaction with an additional monomer capable of further reaction with an additional monomer in the synthesis of an oligomeric ligand.

10. The method of claim 9, wherein the first procedure further comprises successive binding of additional monomers to provide an oligomeric ligand bound to the solid support.

11. The method of claim 10, wherein the oligomeric ligand is an oligonucleotide.

12. The method of claim 10, wherein the oligomeric ligand is an oligopeptide.

13. The method of claim 10, wherein the oligomeric ligand is a peptide mimetic.

14. The method of claim 12, wherein the peptide mimetic is an oligopeptoid.

15. The method of claim 10, wherein the oligomeric ligand is an oligosaccharide.

16. The method of claim 7, wherein the first procedure further comprises chemically modifying the organic compound bound to the solid support.

17. The method of claim 8, wherein the second procedure comprises contacting a sample with the support-bound ligand capable of binding to component therein, in an aqueous medium.

18. The method of claim 10, wherein the second procedure comprises contacting a sample with the support-bound oligomeric ligand capable of binding to component therein, in an aqueous medium.

19. The method of claim 8, wherein a plurality of chemically distinct ligands are provided on the support surface, and the second procedure comprises identifying a ligand of interest by screening for said ligand of interest in an aqueous medium.

20. The method of claim 10, wherein a plurality of chemically distinct oligomeric ligands are provided on the support surface, and the second procedure comprises identifying an oligomeric ligand of interest by screening for said oligomeric ligand of interest in an aqueous medium.

21. The method of claim 8, wherein the second procedure involves chromatographic separation.

22. The method of claim 10, wherein the second procedure involves chromatographic separation.

23. A method for making and using a combinatorial library of chemically distinct ligands on a single support, comprising:

(a) providing a solid support having a surface bearing a plurality of reactive hydrophilic moieties, such that the support surface is compatible with aqueous media;

(b) protecting a fraction of the reactive hydrophilic moieties and the remaining fraction of hydrophilic moieties are unprotected, to provide the surface in a hydrophobic form so that it is compatible with organic media;

(c) conducting successive chemical reactions carried out in an organic medium to provide a plurality of chemically distinct ligands bound to the unprotected hydrophilic sites;

(d) deprotecting the protected fraction of reactive hydrophilic moieties to regenerate the surface in a hydrophilic form; and

(e) without cleaving said ligands from the support, conducting successive screening procedures in an aqueous medium to identify one or more support-bound ligands of interest.

24. The method of claim 23, wherein step (c) comprises solid-phase organic synthesis of an oligonucleotide, and oligopeptide, and oligopeptoid, or an oligosaccharide.

25. The method of claim 23, wherein steps (c), (d) and (e) are repeated one or more times.

26. The method of claim 24, wherein steps (c), (d) and (e) are repeated one or more times.

27. A method for making a substrate which is useful for conducting chemical processes involving a first procedure which is conducted in an organic medium and a second procedure which is conducted in an aqueous medium, comprising:

(a) providing a solid support having a surface capable of being functionalized; and

(b) covalently binding chemical moieties to the support surface in a synthetic procedure involving one or more chemical reactions, to provide a plurality of reactive hydrophilic sides on the support surface situated relative to each other in a manner enabling protection of only a predetermined fraction of the sites using a single protecting group.

28. The method of claim 27, wherein the reactive hydrophilic sites are hydroxyl groups.

29. The method of claim 27, wherein the hydroxyl groups are situated such that three hydroxyl groups are present at the terminus of a single linking moiety covalently bound to the support surface, and the single protecting group is a diol-protecting group.

30. The substrate prepared by the method of claim 27.

31. The substrate prepared by the method of claim 28.

32. The substrate prepared by the method of claim 29.

33. A reaction apparatus for the use of a solid support to conduct a first procedure which is conducted in an organic medium and a second procedure which is conducted in an aqueous medium, comprising

a vessel; and

a solid support comprising a plurality of reactive hydrophilic moieties wherein a fraction of the hydrophilic groups on the support are protected to provide the surface in a hydrophobic form that is compatible with organic media and capable of being deprotected to provide a hydrophilic surface that is compatible with aqueous media.

34. The reaction apparatus of claim 33, wherein the hydrophilic groups are selected so that they are uncharged at neutral pH.

35. The reaction apparatus of claim 34, wherein the hydrophilic groups are selected from the group consisting of hydroxyl, carboxyl, thiol, amines, and mixtures thereof.

36. The reaction apparatus of claim 35, wherein the hydrophilic groups are hydroxyl groups.

37. The reaction apparatus of claim 36, wherein the organic compound is a monomer capable of further reaction with an additional monomer in the synthesis of an oligomeric ligand.

38. The reaction apparatus of claim 37, wherein the oligomeric ligand is a peptide mimetic.

39. The reaction apparatus of claim 38, wherein the peptide mimetic is an oligopeptoid.

40. A method of screening a library of compounds involving a first procedure which is conducted in an organic medium and a second procedure which is conducted an aqueous medium, comprising

(a) providing a reaction apparatus that comprises

(i) a vessel;

(ii) a solid support comprising a plurality of reactive hydrophilic moieties such that the support surface is compatible with aqueous media,

wherein a fraction of the reactive hydrophilic moieties are protected and the remaining fraction of hydrophilic moieties are unprotected, so that the protected groups provide the surface in a hydrophobic form that is compatible with organic media;

(b) conducting a first procedure in an organic medium with the hydrophobic surface provided in step (a);

(c) deprotecting the protected fraction of reactive hydrophilic moieties to regenerate the surface in a hydrophilic form that is compatible with aqueous media; and

(d) conducting a second procedure in an aqueous medium with the hydrophilic surface provided in step (c).

41. The method of claim 40, wherein the second procedure comprises contacting the solid support with a sample comprising a library of organic molecules.

42. The method of claim 41, wherein the first procedure comprises synthesizing a library of organic molecules and the second procedure comprises conducting successive screening procedures in an aqueous medium to identify one or more support-bound ligands of interest.

43. A method of screening a plurality of surface-modified solid supports, comprising

(a) providing a reaction apparatus that comprises

(i) a plurality of reaction vessels;

(ii) contained in each reaction vessel, a solid support comprising a plurality of reactive hydrophilic moieties, such that the support surface is compatible with aqueous media,

wherein a fraction of the reactive hydrophilic moieties are protected and the remaining hydrophilic moieties are unprotected, such that the protected groups provide the surface in a hydrophobic form that is compatible with organic media;

(b) synthesizing a library of organic molecules on the hydrophobic surface provided in step (a), in an organic medium;

(c) deprotecting the protected fraction of reactive hydrophilic moieties to regenerate the surface in a hydrophilic form that is compatible with aqueous media; and

(d) conducting successive screening procedures in an aqueous medium with the hydrophilic surface provided in step (c), to identify one or more contained solid supports of interest.

44. A method for chromatographic separation involving a first procedure which is conducted in an organic medium and a second procedure which is conducted an aqueous medium, comprising

(a) providing a reaction apparatus that comprises

(i) a vessel; and

(ii) a solid support comprising a plurality of reactive hydrophilic moieties, such that the support surface is compatible with aqueous media,

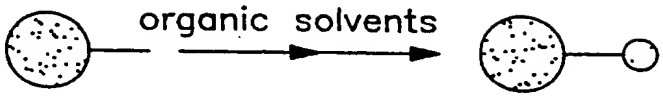
wherein a fraction of the reactive hydrophilic moieties are protected and the remaining fraction of hydrophilic moieties unprotected, so that the protected groups provide the surface in a hydrophobic form that is compatible with organic media;

(b) conducting the first procedure in an organic medium with the hydrophobic surface provided in step (a);

(c) deprotecting the protected fraction of reactive hydrophilic moieties to regenerate the surface in a hydrophilic form that is compatible with an aqueous media; and

(d) conducting the second procedure in an aqueous medium with the hydrophilic surface provided in step (c).

1. Synthesis in organic solvents



2. Screening in aqueous solution

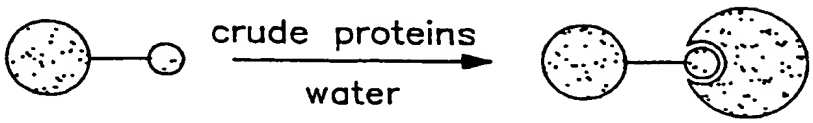


FIG. 1

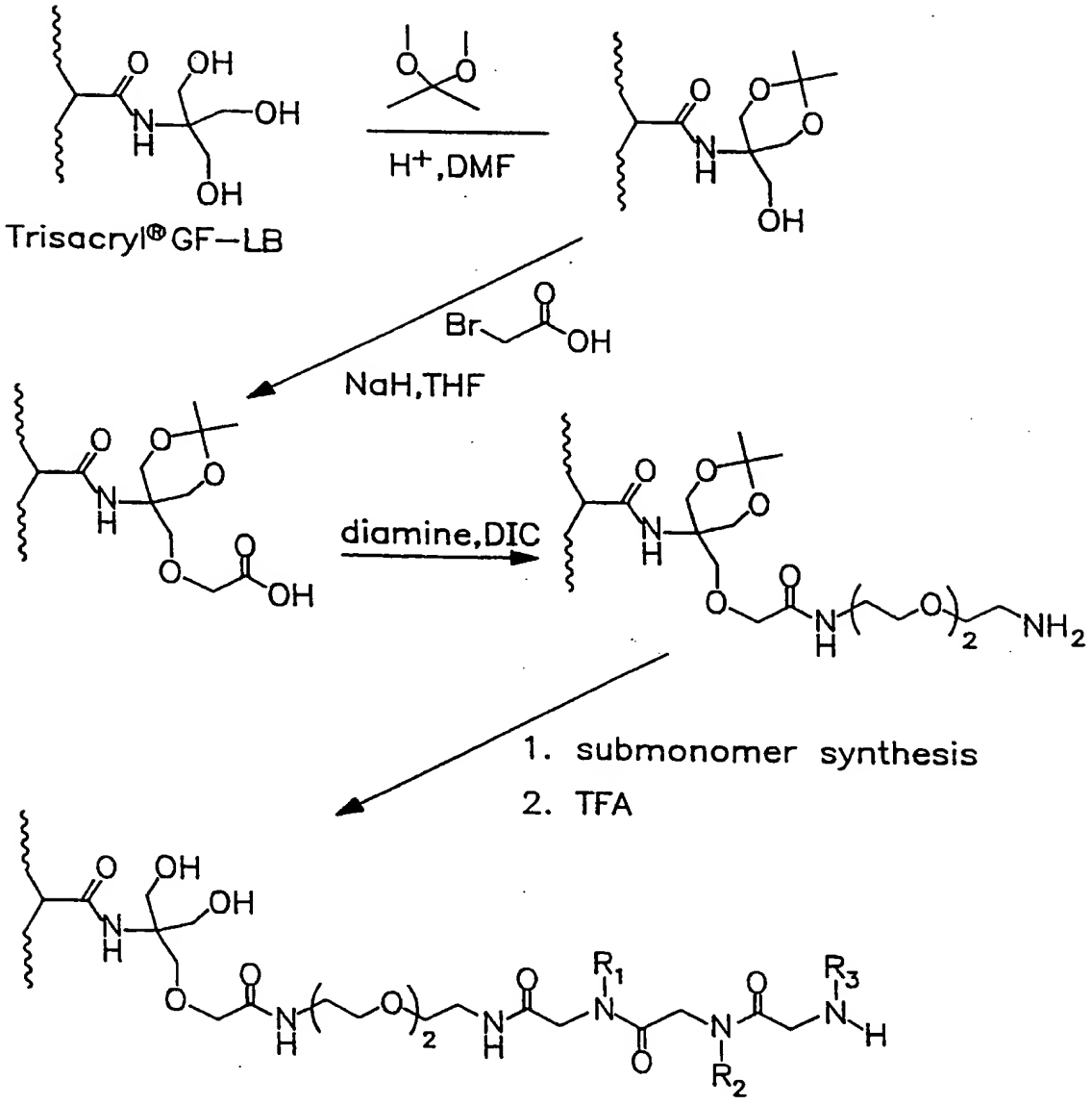


FIG. 2

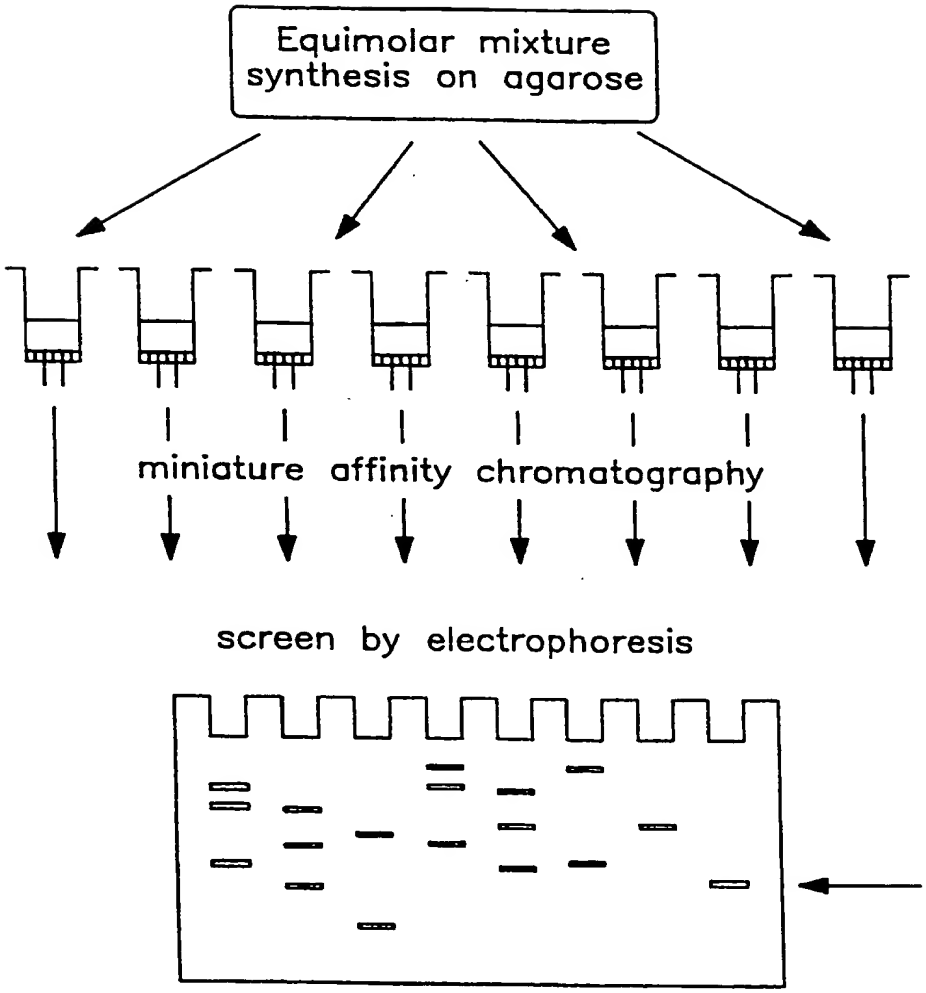


FIG. 3

INTERNATIONAL SEARCH REPORT

Inter. mal Application No
PCT/US 98/06065

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K1/04 C07H21/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07H

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R ARSHADY & F FALLAH: "Amphiphilic gels for peptide synthesis" JOURNAL OF POLYMER SCIENCE, POLYMER CHEMISTRY EDITION., vol. 30, no. 8, 1992, pages 1705-1716, XP000286978 NEW YORK US see the whole document ---	1
X	WO 94 27719 A (T M BRENNAN) 8 December 1994 see example 2 ---	27
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publicationdate of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of theinternational search	Date of mailing of the international search report
19 August 1998	01/09/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Masturzo, P

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INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/US 98/06065

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 123, no. 13, 25 September 1995 Columbus, Ohio, US; abstract no. 170151, XP002074984 & L WINTHER ET AL.: " Hydrophlic film supports for use in peptide synthesis and assays" INNOVAT. PERSPECT. SOLID PHASE SYNTH. COLLECT. PAP., INT. SYMP., 3RD, 1993, pages 705-706, see abstract	1
X	CHEMICAL ABSTRACTS, vol. 102, no. 6, 11 February 1985 Columbus, Ohio, US; abstract no. 46635, R ARSHADY: "A new synthetic approach for the preparation of polymer supports based on beaded copolymers of styren and 2,4,5-trichlorophenyl acrylate: synthesis and swelling behavior off poly(styrene-co-acrylamide) resins " XP002074985 & MAKROMOL. CHEM., vol. 185, no. 11, 1984, pages 2387-2400, see abstract	1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 98/06065

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9427719 A	08-12-1994	US 5474796 A	12-12-1995
		AT 156034 T	15-08-1997
		CA 2163781 A	08-12-1994
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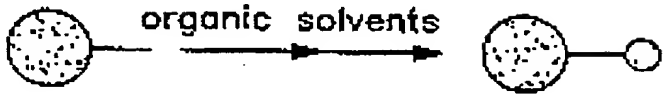
		DE 69404657 T	18-12-1997
		EP 0703825 A	03-04-1996
		JP 9500568 T	21-01-1997

1. Synthesis in organic solvents



2. Screening in aqueous solution

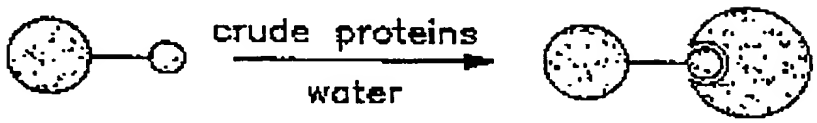


FIG. 1

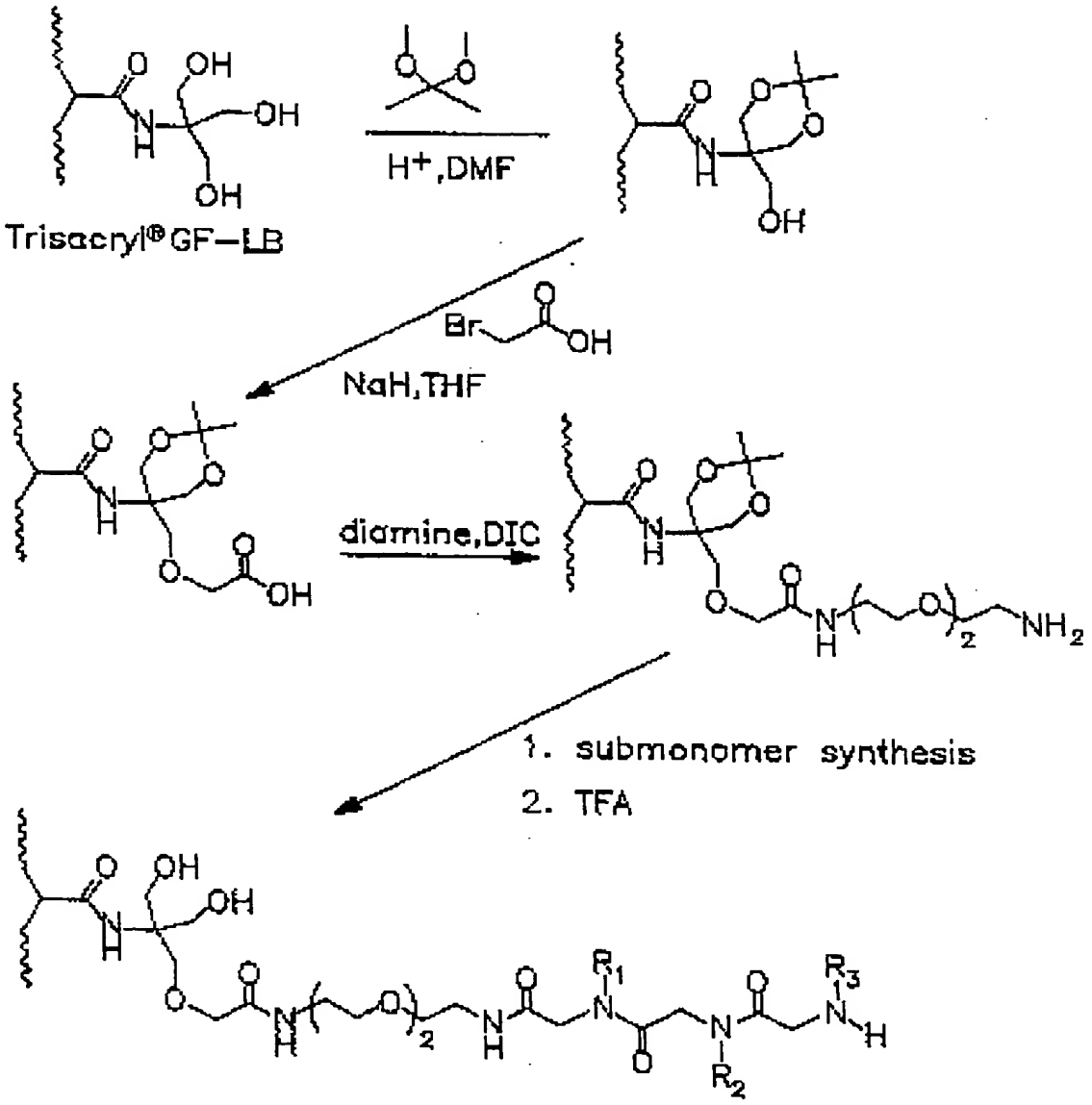


FIG. 2

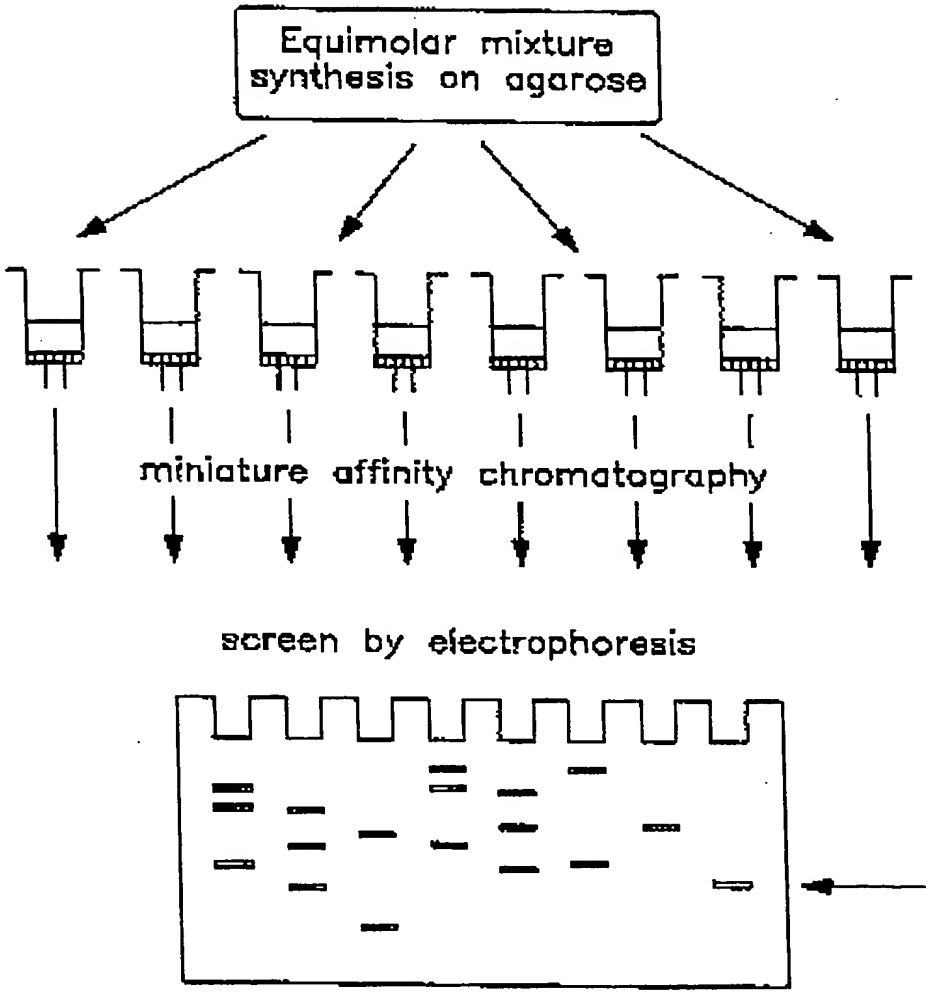


FIG. 3